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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

VIRGINIE LOUVAIN, ET AL : EXAMINER: TSAY, M.M..

SERIAL NO: 10/518,390 :

FILED: OCTOBER 25, 2005 : ART UNIT: 1656

FOR: THROMBIN CLEAVABLE FACTOR X ANALOGUES:

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

Sir:

Now comes Bernard Le Bonniec who deposes and states that:

1. I am a graduate of Université Paris 6 and received my PhD degree in the field of Biochemistry in the year 1986.

2. I have been employed by INSERM, for 13 years in the field of Thrombosis and Haemostasis.

3. I am a named inventor of the above-identified application.

4. I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same.

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5. The following experiments were carried out by me or under my direct supervision and control.

6. The Examples of the present application show that the factor X analogues according to the present invention are cleaved by thrombin and generate amidolytic activity.

In Example 1, beginning on page 9 of the specification, the construction of expression vectors for factor X analogues was disclosed. Specifically, several analogues of factor X were produced, which are as follows (see Table I on page 10 of the specification):

	Factor X analogue	**Sequence P_3-P_2-P_1-P'_1-P'_2-P'_3**
SEQ ID No 7	GDX-IVG	VPR-IVG
SEQ ID No 8	GDX-IFG	VPR-IFG
SEQ ID No 9	GDX-AVG	VPR-AVG
SEQ ID No 10	GDX-IFR	VPR-IFR
SEQ ID No 11	GDX-SVG	VPR-SVG
SEQ ID No 12	GDX-SFR	VPR-SFR

In Example 4 of the present application (see pages 18-21 of the specification), the rate of cleavage of the factor X analogues by thrombin was evaluated, depending on whether or not this cleavage generates a detectable amidolytic activity. Those experiments also made possible the measurement of the amidolytic activity generated by the activated factor X analogues.

The experiment is a Michaelis Menten kinetics experiment, wherein:

- **K_m** is a constant that is equal to the substrate concentration at which an enzyme reaction proceeds at half the maximum velocity and is associated with the affinity of the enzyme (thrombin) for substrate (the zymogen derived from factor X) ;
- **k_{cat}** gives a direct measure of the catalytic production of product under optimum

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conditions ; and

- **k_{cat}/K_m** represents a measure of enzyme efficiency.

The rate constant was measured, which is directly proportional to the specificity constant (k_{cat}/K_m) of the enzyme (thrombin) for its substrate (the zymogen derived from factor X).

Table V of the present application (see page 21) puts in light the following facts:

- GDX-SVG, GDX-IVG, GDX-IFG and GDX-IFR are cleaved by thrombin but the reaction is too slow for it to be possible to estimate the value of the k_{cat}/K_m ;
- GDX-SFR analogue is cleaved very rapidly but does not generate detectable amidolytic activity ($k_{cat}/K_m=4.10^3\ M^{-1}.s^{-1}$) ; and
- GDX-AVG analogue is cleaved by thrombin and has readily detectable amidolytic activity ($k_{cat}/K_m=1.10^2\ M^{-1}.s^{-1}$).

This experiment corroborates the fact that VPR-SFR is highly favorable for cleavage by thrombin as described in the previous art.

Moreover, this experiment clearly evidences that VPR-AVG analogue is cleaved by thrombin, in a lesser extent than VPR-SFR, but surprisingly provides a higher amidolytic activity than the others factor X analogues.

7. The Examples of the present application show that the activated form of GDX-AVG analogue interacts with factor Va.

In Example 5 of the present application (see pages 21-38) the activation of prothrombin (which is naturally activated by factor Va and activated factor X).

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This experiment clearly illustrates the fact that the addition of factor Va restores the catalytic activity of the activated form of GDX-AVG analogue. In addition, this experiment shows that factor Va does not provide such results with any of the others factor X analogues.

This indisputably indicates that the activated form of GDX-AVG analogue interacts with factor Va, and thus activates prothrombin.

8. The Examples of the present application show that the activated form of GDX-AVG analogue has a higher half life than its native homologue.

In Example 5, the ability of each activated form of the factor X analogues to form a stable covalent complex with antithrombin was determined. For this experiment, the k_{on} of the interaction of antithrombin with the activated forms of the factor X analogue was ascertained.

Physiologically, antithrombin is an inhibitor of the activated form of factor X and the value of its k_{on} for the interaction with activated form of factor X is about 10^4 $M^{-1}.s^{-1}$.

In this experiment, a lower value of the k_{on} of a factor X analogue suggests that its interaction with antithrombin is less effective, and thus that said analogue remains active for longer.

The results of this experiment are summarized in Table IX of the present application (see page 36):

- in absence of heparin, the value of k_{on} of the antithrombin for the activated form of GDX-AVG analogue is about 10 $M^{-1}.s^{-1}$, i.e. more than 1000 times less than that of its non mutated homologue ($k_{on}=1.2.10^4$ $M^{-1}.s^{-1}$), and 10 to 100 times less than the k_{on} values of the others factor X analogues; and
- in presence of heparin, the value of the k_{on} of the antithrombin for the activated form of GDX-AVG ($k_{on}=3.01.10^2$ $M^{-1}.s^{-1}$) is far lower than for the others factor X analogues.

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This observation undoubtedly indicates that, after activation, the GDX-AVG analogue remains active for longer than its non-mutated homologue, which prolongs the procoagulant action of the analogue and therefore considerably reinforces its anti-haemophilic properties.

To confirm this hypothesis, Applicants determined the plasma half life of the activated form of the factor X analogues by measuring their residual activity after incubation for a varying amount of time in a pool of normal human plasmas.

The results are summarized in Table X of the present application (see page 38):

- in presence of heparin, the half life of activated GDX-AVG analogue is about 5 minutes and 30 seconds, whereas the half lives of the others analogues are less than 30 seconds;
- in the absence of heparin, the half life of the activated form of GDX-AVG analogue is notably extended and is about 55 times longer that the others activated factor X analogues.

The foregoing observations would not be apparent to or even expected by the skilled artisan prior to the present application on the basis of his general knowledge or in view of the teachings of Himmelspach et al (US 6,573,071).

9. The Examples of the present application show that the activated form of GDX-AVG analogue has a procoagulant activity.

The procoagulant activity of the activated forms of the factor X analogues was tested. The procoagulant activity of the factor X analogues is compared with that of the normal homologue lacking Gla domain (GD-FX).

Table XI of the present application (see page 40) shows that the activated form of GDX-AVG analogue shortens the clotting time as much as the activated form of the GD-FX analogue, which is not true for the other activated factor X analogues.

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This result corroborates the fact that the GDX-AVG analogue clearly has a procoagulant action, unlike the others factor X analogues.

This result is confirmed by Fig. 4 of the present application which compares the procoagulant effect of the GDX-AVG analogue with the GD-FX analogue in factor VIII-depleted (4A) or factor IX-depleted (4B) plasma.

Fig. 4 shows that in the presence of GDX-AVG analogue, the clotting time is shorter than in presence of GD-FX, which undeniably confirms that GDX-AVG analogue is more active than GD-FX analogue.

The fact that GDX-AVG analogue is more active than the GD-FX indicates that an amplification of thrombin generation has indeed taken place in the presence of GDX-AVG.

Example 5 clearly shows that the GDX-AVG analogue leads to a production of at least 26 times more activated forms of factor X.

10. In conclusion, the foregoing clearly shows that:

    1) The GDX-AVG analogue of factor X is efficiently cleaved by thrombin, resulting in the activated form of GDX-AVG analogue;

    2) the activated form of GDX-AVG analogue provides a high amidolytic activity;

    3) the activated form of GDX-AVG analogue interacts with factor Va and activates prothrombin;

    4) the activated form of GDX-AVG analogue has a higher half time than native activated factor X;

    5) the activated form of GDX-AVG has a procoagulant activity; and

    6) the activated form of GDX-AVG analogue establishes an autoamplification of thrombin generation.

11. The foregoing evidence clearly establishes that the present invention of an analogue of factor X which has the unexpected result of bypassing the deficient steps of the clotting

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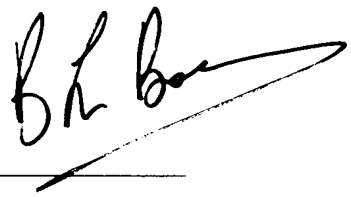
cascade. This invention was borne by overcoming the drawbacks of the therapeutic approaches in place prior to the present invention but also establish auto-amplification of thrombin generation in subject suffering from haemophilia. There is no disclosure or suggestion in Himmelspach et al (US 6,573,071) to select the very specific analogue with a sequence VPR-AVG in the activation peptide, among all factor X analogues disclosed therein. As such, there is nothing expected about the foregoing results when referring to Himmelspach et al (US 6,573,071).

12. I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

13. Further Declarant saith not



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Date April 19, 2010

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